



## Original article

# Immunological effect of *Moringa Oleifera* leaf extract on vaccinated and non-vaccinated Hubbard chickens experimentally infected with Newcastle virus



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## ABSTRACT

In veterinary medicine plant based medicine is achieving a huge importance worldwide. This research was subjected to rectify the hydrophilic *Moringa Oleifera* alcoholic leaves extract could improve the immune system in vaccinated and non-vaccinated broiler Hubbard chickens experimentally exposed to Newcastle disease (ND) virus. Seventy five chicks with age one day old were splitted randomly into five groups equally in distribution with fifteen chick in each group. Group I was untreated unvaccinated (control negative group) while group IV was infected group with NDV (control positive group). The experimental Groups II and V were given daily oral treatment of hydrophilic alcoholic leaves extract of *M. oleifera* at 200 mg/kg body weight until day 21 of age while groups III and V were ND vaccinated with La Sota strain of ND vaccines. The four groups (II, III, IV, V) were infected with ND virus velogenic strain (VNDV) on day 21. Following to infection, Monitoring of birds were done daily for clinical signs, postmortem examination, morbidity and mortality. Cellular, humeral immune response and phagocytic activity were evaluated and the data were statistically analyzed using (SPSS). Total and differential cell numbers as well as Haemagglutination inhibition (HI) titre increased in the extract treated and vaccinated group which give total protection against NDV much more than treated and unvaccinated group. As a result it could be recommended to use *M. Oleifera* extract from the first day of rearing in Hubbard chicken with ND vaccination program as a prophylactic treatment in protection of birds against ND infection.

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## 1. Introduction

The poultry industry is one of the world's most fundamental agricultural sectors. Poultry provides humans with an excellent source of animal protein in the form of eggs and meat. Poultry meat accounts for 20% of total daily fish and animal protein consumption per capita (Alders et al., 2018). Poultry production showed ecumenical increase from backyard farm to advanced

poultry farm including commercial meat and egg production. Diseases result from viral agents in poultry ranches cause a tremendous economic losses. Newcastle disease is a highly contagious and infectious viral disease of domestic, aviary, caged, and wild birds that causes severe morbidity, mortality, economic losses, and risks to the global chicken industry (Rehman et al., 2020). Newcastle disease virus is mainly controlled by vaccination but some challenges particularly in rural areas with pauper ranches as high vaccines costs, unavailability of cold chain systems used these vaccines in addition to small flock sizes with multi-aged birds may effect on the success of vaccination control system (El-Masry et al., 2019) Vaccination failures and outbreaks of the Newcastle disease have also been happened as a result of neutralization of the vaccine virus at the time of vaccination by maternal antibodies as well as the presence of antiemetic variants in the field that are kept in continuous circulation (Garba et al., 2013; Hegazy et al., 2021). Ameliorating the immunogenicity of the vaccines by

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the enforcement of natural herbal plants, for example, are complementing ways that may be a pretty way to conquer such infectious diseases. Some studies reported the enhancement in the B and T cell performance and antibody response after co-administration of medicinal plants extracts with vaccine (Hu et al., 2003; Sun et al., 2007). The use of curative plants is progressively earning value as products of natural sources. They offer medicinal value for a variety of ailments and are considered one of the most essential components of indigenous medical systems that have survived in developing countries (Abd-Rabou et al., 2017; Abdel Rahman et al., 2019). One of these plants is *Moringa Oleifera lam* belongs to family Moringaceae. It is now widely distributed throughout subtropical and tropical areas in the world. It is Native to India and contains various active substances as phytochemicals, phenolics, vitamins, amino acids macronutrients, and micronutrients which have antioxidant, antiviral activity, anti-inflammatory, antimicrobial and use to expel intestinal worms also has an immunostimulatory effects (Brilhante et al., 2017; Desoky et al., 2019). Therefore the hypothesis of this study is to detect the antiviral and protective effect of *Moringa Oleifera* leaf extract in broiler Hubbard chickens against velogenic strain of Newcastle virus and study the effect of this leaf meal on the action of Newcastle virus vaccine.

## 2. Material and methods

### 2.1. Birds and handling

A total of seventy-five one day old commercial chickens obtained from El-Bana Company were applied and put in separate units until the beginning of the experimental study. The chickens were divided in five experimental groups in Negative Pressure Isolators, under bio-safety conditions in experimental units in Faculty of Veterinary Medicine, Zagazig University. Birds were fed on commercial starter, pelleted ration and kept under the managerial conditions as lightening was provided 23hr light and 1 h dark with cyclic temperatures (minimum 24°C, maximum 32 °C). Feed and water were provided ad libitum. All experimental chickens were used according to the Committee of Animal Welfare and Research Ethics (protocol #ZU-IACU/2/F/9/2018) in Zagazig University, Egypt.

### 2.2. NDV propagation and titration

NDV were utilized in the present study was velogenic NDV class II, genotype VIIId (A/chicken/Egypt/AB3/2018) under an accession number of MK968881 was isolated in department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt. Prior to use in experimental infection, virus propagation and titration were carried in embryonated chicken eggs according to (Aldous and Alexander, 2001; Sheble and Reda, 1976), as well as, virus titers were calculated according to (Reed and Muench, 1938). The inoculation dose was  $10^6$ (ELD50) of 0.1 mL of infectious allantoic fluid. Live vaccines used in this our study including LaSota (LS) (BAL-ND (B1 type LaSota strain live vaccine), G.O.V.s Batch no:L 141/1 NDV strain.

### 2.3. Preparation of hydro-alcoholic extract of *Moringa Oleifera*

From the middle aged green trees in agricultural orchards of Faculty of agriculture, Zagazig University the dried leaves of *Moringa Oleifera* were harvested and grounded into fine coarse powder by hand and mortem. The leaves powder (200 g) was soaked in ethanol 95% (1L): water (1L) 1:1 at 33c for 24 h. The mixture was subjected to filtration using a Watman grade 1 filter paper

then the solvent was evaporated and the extract was lyophilized. The extracts were stored in a dark bottle at  $-4$  °C until used. The crude extract was suspended in distilled water (Elrys et al., 2019; Prabsattroo et al., 2012) and by using standard methods according to (Trease and Evans, 1972) Phytochemical analyses of the extracts were performed by Elangovan (2014).

### 2.4. Experimental infection and sampling

At one day of age, the birds should divided into five equal groups randomly (15 chicks/group). From the other groups, group I (control negative) and group IV (control positive) were isolated Groups II&V were treated The treatment was with the *Moringa Oleifera* leaves extract 200 mg/kg body weight orally used in drinking water daily for three weeks while groups III&V were vaccinated. At 21 day of age all the groups were challenged intraocular with dose of 0.2 of  $10^6$  of vNDV. After infection, clinical signs were monitored daily. For viral shedding evaluation, oropharyngeal and cloacal swabs were collected at 3rd 5th 7th and 9th day post infection, placed in sterile phosphate buffer saline and stored at  $-70$  °C until used. Blood samples were collected for hematology at 14, 21 and 28 days of ages and at 21 day of age before inoculation, 3dpi and 5dpi for phagocytic activity. Blood centrifugation was used to separate the serum at 14, 21 day post vaccination and at 14 day post challenge were collected and stored at  $-20$  °C until used for immune response evaluation by haemagglutination test. The birds were vaccinated with LaSota (BAL-ND) (B1 type LaSota strain live vaccine) at 15 day of age eye drop route.

### 2.5. Serology

The antigen titre for running the HI test was determined by standard HA technique using La Sota ND vaccine as antigen according to (Alexander and Senne, 2003). The reciprocal of the highest dilution of the La Sota ND antigen causing 100% agglutination of an equal volume of standardized RBCs was taken as the HA titre of the antigen. The HI titres were determined, also by the method of (Beard, 1989). The HI titers were reciprocal of the highest dilutions of the sera at which 100% RBC HI occurred. The geometric mean titre (GMT) was calculated according to (Villegas, 1998).

### 2.6. Hematology

Cellular immune response was assessed by detecting the total leukocytic counts of white blood cells (W BC) which obtained according to standard methods using improved Neubauer hemocytometer and differential leukocytic count which done in stained thin blood smears Coles (1986).

### 2.7. Phagocytic activity

Blood was collected from three birds weekly before challenge and week after challenge in a test tube containing EDTA anticoagulant then send to Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQP), Zagazig to detect the Phagocytic index of Macrophage cells before and after challenge.

### 2.8. Virus shedding

RNA was extracted from cloacal and oropharyngeal swabs that collected at 3rd, 5th, 7th and 9th PI according to the manufacturer's protocol using the QIamp Viral RNA Mini Kit (QIAGEN, USA), Quantitative real-time RT-PCR (qRT-PCR) was done by (Wise et al., 2004). Briefly, a one-step qRT-PCR using sequence-specific probes for gene expression analysis was done according to the instructions of the manufacturer (QIAGEN, The Netherlands)

**Table 1**  
The primers used for detection of M gene of Newcastle disease virus.

Virus	Gene	Primer/probe sequence 5'-3'	Amplified Segment (bp)	Ref
ND	M	<b>M + 4100</b> AGTGATGTGCTCGGACCTTC-3' <b>M - 4220</b> CCTGAGGAGAGGCATTTGCTA-3' <b>M + 4169</b> [FAM]TTCTCTAGCAGTGGGACAGCCTGC[TAMRA]-3'	121	Wise et al., 2004

and using the ABI 7500 Real-Time PCR system (Applied Biosystems, Carlsbad, CA). Primers and probes targeting M gene of Newcastle virus indicated in (Table 1). For viral quantification with viral RNA extracted from the titrated challenge virus a standard curve was established.

2.9. Statistical analysis

SPSS software (v.16) and one-way analysis of variance (ANOVA) was used for data analysis, the significance differences between the mean values were calculated using Tukey's HSD multiple comparison tests. The alpha level for determination of significance was 0.05. Means in the same column followed by different letters were different significantly and the highest value was represented by the letter (a).

**Table 2**  
Results of phytochemical analyses of *Moringa oleifera*.

Compounds	Presence in methanol extract
Alkaloid	+
Saponin	++
Tannins	-
Glycoside	++
Flavonoid	+
Steroid	+++
Terpenes	-
Fats and Oil	+
Reducing Sugar	++

+ present in low concentration, ++ present with moderate concentration, +++ present in high concentration and - mean absent.

**Table 3**  
The HI antibody titers in vaccinated chickens with Newcastle vaccine before and after challenge and vaccination.

Groups	14 day post vaccination	21 day post vaccination	14 day post challenge
III	3.4 ± 0.11	6.4 ± 0.16	7.4 ± 0.17
V	6.5 ± 0.16	7.6 ± 0.11	8.0 ± 0.11

**Table 4**  
Effect of oral administration of *Moringa* hydro alcoholic extract (200 mg/kg b.wt) once daily for 21 successive days on total leukocytic count, lymphocyte, neutrophil, basophil and eosinophil.

TIME	Groups parameters	I	II	III	IV	V
		Control negative	Plant + Virus	Vaccine + Virus	Control positive	Vaccine + Virus + Plant
At zero time	TLC(103/μl)	79.2 ± 0.14 d	80.04 ± 0.02 c	82.6 ± 0.11 a	81.4 ± 0.11 b	82.4 ± 0.23 a
	lymphocyte	70.5 ± 0.14 d	72.3 ± 0.05 b	73.1 ± 0.05 a	72.1 ± 0.08 b	71.1 ± 0.08 c
	Neutrophil	2.1 ± 0.28 c	2.8 ± 0.20 a, b	3.2 ± 0.14 a	2.5 ± .18 b c	3.1 ± 0.05 a b
	basophil	5.1 ± 0.05 a	5.3 ± 0.05 a	5.2 ± 0.05 a	5.1 ± 0.29 a	5.2 ± 0.05 a
	eosinophil	2.1 ± 0.05 b	2.3 ± 0.05 b	2.6 ± 0.05 a	2.2 ± 0.05 b	2.2 ± 0.12 b
1st week	TLC (103/μl)	79.30 ± 0.05 a	60.4 ± 0.14 c	61.4 ± 0.05 c	40.2 ± 0.12 d	75.5 ± 1.2 b
	lymphocyte	72.2 ± 0.11 a	60.8 ± 0.03 c	61.2 ± 0.14 c	4 5.1 ± 0.08 d	70.2 ± 0.60 b
	Neutrophil	2.2 ± 0.05 a	1.3 ± 0.08 c	1.3 ± 0.08 c	0.6 ± 0.04 d	1.9 ± 0.05 b
	basophil	5.2 ± 0.15 a	3.2 ± 0.12 b	3.1 ± 0.08 b	1.9 ± 0.05 c	5.00 ± 0.05 a
	eosinophil	2.5 ± 0.21 a	1.3 ± 0.08 b	1.4 ± 0.05 b	0.6 ± 0.02 c	2.2 ± 0.15 a
2nd week	TLC (103/μl)	80.4 ± 0.21 a	66.06 ± 0.29 c	66.6 ± 0.43 c	38.86 ± 0.47 d	78.6 ± 0.35 b
	lymphocyte	72.4 ± 0.2 a	63.5 ± 0.14 c	63.8 ± 0.05 c	43.1 ± 0.05 d	70.9 ± 0.12 b
	Neutrophil	2.3 ± 0.12 a	1.4 ± 0.05 c	1.3 ± 0.06 c	0.4 ± 0.1 d	1.9 ± 0.05 b
	basophil	5.2 ± 0.14 a	4.6 ± 0.08 b	4.6 ± 0.06 b	1.7 ± 0.05 c	5.1 ± 0.05 a
	eosinophil	2.2 ± 0.2 a	1.7 ± 0.08 b,c	1.6 ± 0.05 c	0.5 ± 0.02 d	2.00 ± 0.05 a,b

3. Results

3.1. Phytochemical analysis

By qualitative phytochemical tests the extract was analyzed into alkaloid, glycoside, saponin, flavonoid, fats, steroid, reducing sugar and oil. The degree of concentrations of glycoside, saponin, reducing sugars and steroid were high in the the methanolic extract of *M. oleifera* while terpenes and tannins were not appeared as shown in (Table 2).

3.2. Clinical signs and postmortem lesions

On 2 day post-infection (P.I), slight conjunctivitis, depression, ruffled feathers, watery diarrhea, anorexia, closed eyes with watery secretion drilled from the mouth were showed in addition to respiratory signs in form of dyspnea, sneezing and nasal discharge. These signs became clearer with time so that at 3dpi were obviously observed. Neurological signs were detected at 6 day post infection in infected group. On day 3PI, group II had a morbidity rate of 35%, group III had a rate of 16%, group V had a rate of 0%, and group IV had a rate of 100%. Morbidity was 36% in group II, 7% in group III, and 0% in group V on day 6 PI. No mortalities were recorded in all groups except in infected group with Newcastle the mortality was 100% within 7 day post infection. The Post mortem examination showed slight congestion in thigh and breast muscle with slight petechial hemorrhage in trachea in group II, III, IV and V were 20%, 40%, 60% and 0% respectively and there was hemorrhage between oesophagus and proventriculus in group II, III and V were 0% except group IV was 50%.The cecal tonsils were also enlarged and hemorrhagic with percentage 20%, 10% 65% and 0% in group II, III, IV and V receptively.

3.3. Serology

All pre-vaccination sera collected before virus inoculation were negative for ND antibodies in the Haemagglutination test. In Experimental group II, treated and non-vaccinated. The mean HI

**Table 5**  
The phagocytic index of the birds experimentally treated with *Moringa Oleifera* and Newcastle vaccine.

Groups	Phagocytic index				
	Control negative Neither infected nor treated	Treated group with <i>M. Olifera</i> and infected	Vaccinated and infected group	Control positive infected not vaccinated	Vaccinated treated and infected
At zero time	0.45 ± 0.03 <sup>ab</sup>	0.44 ± .05 <sup>b</sup>	0.45 ± .05 <sup>ba</sup>	0.46 ± .05 <sup>a</sup>	0.46 ± 0.03 <sup>ab</sup>
3rd day	0.46 ± 0.03 <sup>c</sup>	0.64 ± .03 <sup>b</sup>	0.61 ± .03 <sup>c</sup>	0.51 ± .03 <sup>d</sup>	0.82 ± 0.05 <sup>a</sup>
5th day	0.44 ± 0.01 <sup>c</sup>	0.61 ± .01 <sup>b</sup>	0.58 ± .03 <sup>c</sup>	0.49 ± 0.03 <sup>d</sup>	0.79 ± 0.05 <sup>a</sup>

**Table 6**  
The phagocytic percentage of the birds experimentally treated with *Moringa Oleifera* and Newcastle vaccine.

Groups	Phagocytic %				
	Control negative Neither infected nor treated	Treated group with <i>M. Olifera</i> and infected	Vaccinated and infected group	Control positive infected not vaccinated	Vaccinated treated and infected
At zero time	41 %±.3 <sup>b</sup>	41%±.3 <sup>b</sup>	41%±.2 <sup>b</sup>	40%±.3 <sup>b</sup>	42%±0.3 <sup>a</sup>
3rd day	41%±0.3 <sup>e</sup>	46%±.2 <sup>b</sup>	44%±.3 <sup>c</sup>	43%±0.1 <sup>d</sup>	64%±.3 <sup>a</sup>
5th day	40%±0.3 <sup>e</sup>	44%±.1 <sup>b</sup>	42%±.3 <sup>c</sup>	41%±0.3 <sup>d</sup>	57%±.3 <sup>a</sup>

The phagocytic percentage = Number of PMN that have engulfed bacteria /Number of PMN counted.

titers was **5.0 ± 0.6** log2 at 14 day post challenge while in Experimental group III, vaccinated and challenged group the The mean HI titers at 21 days post vaccination was **6.4 ± 0.16** log2 higher than

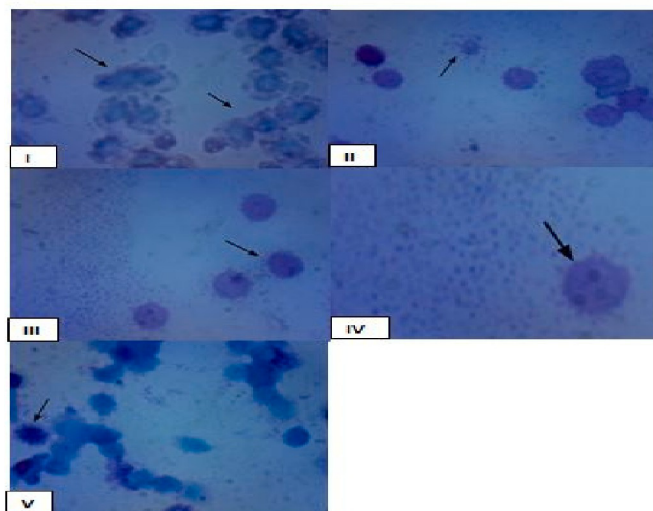
the mean HI titers **3.4 ± 0.11** log2 at 14 days post vaccination but at 14 day post challenge the HI titre was **7.4 ± 0.17**log2. In Experimental group V Mean HI titers were **6.5 ± 0.16** log2 & **7.6 ± 0.11** log2 of chickens immunized with vaccines in combination with *Moringa Oleifera* at 14 and 21 day post vaccination respectively and **8.0 ± 0.11** log2 post challenge. The data were analyzed by HI test (Table 3).

3.4. Hematological parameters

Leukogram was detailed in Table 4. There is a significant decrease in TLC, lymphocyte, neutrophil, basophil and eosinophil in Group IV when compared with group I at 1st and 2nd week. In group V there is a significant increase in TLC, lymphocyte, neutrophil, basophil and eosinophil when compared with group IV at 1st and 2nd week. While there was no significant difference between group (II) and group (III) in the same parameters.

3.5. Phagocytic activity

The result of phagocytic activity is indicated by increasing in the mean number of *Saccharomyces cerevisiae* per macrophage cells as shown in group II due to pretreatment of plant in virus infected group also the same result in group III showing effect of vaccine in increase phagocytic capacity of Macrophage against NDV. In group V showed the highest phagocytic ability of macrophage cells related to synergistic effect of vaccine and plant against NDV. The phagocytic index (PhI) was calculated by the percentage of phagocytes involved in phagocytosis×the mean number of adhered/ingested *Saccharomyces cerevisiae* per phagocytosing cell Tables 5 and 6; Fig. 1.



**Fig. 1. Microscopical examination of phagocytic index of Macrophage cells three days after experiments.** Group (1): showing normal phagocytic ability of Macrophage cells. (control –ve), Group II: showing increase in the mean number of *Saccharomyces cerevisiae* per Macrophage cells due to pretreatment of plant in virus infected group. Group III: showing effect of virus and vaccine in increase phagocytic capacity of Macrophage. Group IV: showing effect of virus presence in the mean number of *Saccharomyces cerevisiae* per Macrophage cells. (control +ve) and Group V: showing the highest phagocytic ability of macrophage cells related to synergistic effect of virus, vaccine and plant.

**Table 7**  
Showed the shedding of the NDV from oropharyngeal and cloacal swabs in experimental infected Hubbard chickens:

Groups	3 days post challenge		5 days post challenge				7 days post challenge		9 days post challenge			
	Log <sub>10</sub> EID <sub>50</sub>	CT	Log <sub>10</sub> EID <sub>50</sub>	CT	Log <sub>10</sub> EID <sub>50</sub>	CT	Log <sub>10</sub> EID <sub>50</sub>	CT	Log <sub>10</sub> EID <sub>50</sub>			
OP <sup>a</sup>	CT	cl <sup>b</sup>	OP <sup>a</sup>	cl <sup>b</sup>	OP <sup>a</sup>	cl <sup>b</sup>	OP <sup>a</sup>	cl <sup>b</sup>	OP <sup>a</sup>	cl <sup>b</sup>		
I	5.2 ± 0.17	4.6 ± 0.15	29.3 ± 1.57	4.0 ± 0.21	2.5 ± 0.05	31.73 ± 0.31	2.0 ± 0.13	1.0 ± 0.18	33.34 ± 0.98	–	–	34.62 ± 2.87
II	5.7 ± 0.31	4.4 ± 0.21	28.7 ± 2.12	4.3 ± 0.31	3.3 ± 0.11	30.21 ± 0.31	2.5 ± 0.05	1.0 ± 0.31	32.0 ± 0.98	–	–	33.7 ± 0.57
III	4.3 ± 0.15	3.9 ± 0.15	31.0 ± 1.64	3.2 ± 0.11	2.5 ± 0.07	32.1 ± 0.18	1.5 ± 0.13	1.0 ± 0.07	33.34 ± 1.47	–	–	34.62 ± 1.89
IV	5.0 ± 0.21	4.3 ± 0.21	30.7 ± 0.98	3.5 ± 0.11	2.9 ± 0.05	32.0 ± 0.31	1.8 ± 0.31	1.0 ± 0.13	33.18 ± 0.57	–	–	34.05 ± 2.87
V	4.9 ± 0.07	4.4 ± 0.21	29.8 ± 1.57	4.0 ± 0.07	3.0 ± 0.11	32.85 ± 0.18	2.0 ± 0.21	1.0 ± 0.13	33.92 ± 1.02	–	–	34.62 ± 1.49

± Means standard error within the same row carrying different superscript were significantly different at P value < 0.05.



### 3.6. Analysis of viral shedding

Cloacal and Oropharyngeal swabs were taken to reveal virus shedding at 3rd, 5th, 7th and 9th days post challenge. The result of virus shedding revealed that oropharyngeal swabs at 3rd day post infection showed the highest value of mean  $\log^{10}$ EID<sub>50</sub> in infected groups of NDV more than in cloacal swabs. Since the mean threshold cycle (Ct) values were observed ranged between (29.3–34.62), all groups shed virus at a comparable level. The mean Ct in Group V were  $29.8 \pm 1.57$ ,  $32.85 \pm 0.18$ ,  $33.92 \pm 1.02$ ,  $34.62 \pm 1.49$  whereas the viral load continued to decrease at 5th, 7th day post challenge. However, NDV RNA levels were dropped at 9 th day post challenge as in Table 7.

## 4. Discussion

Newcastle disease (ND) is a highly contagious viral disease that affects poultry. Newcastle disease (ND) is an avian disease that affects chickens, turkeys, pigeons, and other birds. It is caused by the Newcastle disease virus, which is a filterable virus (NDV). In unvaccinated birds, morbidity and mortality in susceptible birds can reach 90–100% in the severe stages of the disease. Respiratory, digestive, and neurological manifestations characterized the infection. Despite vaccination and therapy programmes, total average losses in poultry owing to ND are currently estimated to be 40–60% (Bello et al., 2018) but vaccination has some demerits as it take long time to produce protective immunity and also the high cost of Vaccines so (Andleeb et al., 2020; Li et al., 2020) found that using medicinal plant extracts as antiviral medicines is a good way for protection the flocks against NDV. Use of medicinal plant extracts as antiviral medicines against NDV is one of the methods to protect flocks against NDV. So in this research we study the effect of *Moringa Oleifera* which is considered one of the medicinal plants which has antiviral properties and play a critical role in the body's immune system's defense against the Newcastle disease virus (Chollom et al., 2012) also it has other merits as it is easily handle and available to the bird breeder with low cost and to compare the protective efficacy of *Moringa Oleifera* extract in chickens with or without NDV vaccination against Newcastle disease virus. Clinical signs in infected groups (depression in appetite, huddle together, greenish diarrhea, respiratory signs and nervous signs) were observed. The same signs recorded by (Alders and Spradbrow, 2001; Okoye et al., 2000).

The gross lesion was as the same reported by Okoye et al. (2000) for vVND. This is because the virulence and the tropism of the virus strain, their immunity and target species (Alexander and Senne, 2003). The lesions were more severe in group IV, followed by groups III and II. The incubation period of experimental Newcastle was ranged between 3 days as reported by Okoye et al. (2000) and from 2 to 16 days as by Hamid et al. (1991) so the morbidity rate was higher in infected and treated unvaccinated group (group II) and in (group IV) infected group the same result recorded by Okoye et al. (2000) who reported the high morbidity of the NDV may reach 100% in unvaccinated birds.

Haemagglutination inhibition test was used to evaluate immunogenicity effect of *Moringa Oleifera* leave extract with and without NDV vaccines. The mean HI titer at 21 day post vaccination in immunized chickens were more greater than the mean HI titer at 14 day post vaccination in sera of chickens. In the Previous studies mentioned at 21 day post vaccination. Vaccinated chickens could be totally protected from highly pathogenic NDV challenge if antibody titers to the challenge virus were equal to or greater than  $4\log_2$ , especially 21 days after immunization (Eze et al., 2014). The mean HI titer of immunized chicken and treated with *Moringa Oleifera* leave extract at 21 day

post vaccination was much higher than 14 day post vaccination neither in vaccinated group nor in *Moringa Oleifera* leave extract group. Two weeks after challenge, vaccinated and treated chickens showed a significant increase in antibody titers. This contradicts the findings of other studies. (Tully et al., 2009), this could be due to a reduction in challenge virus replication in the survivors. The efficacy assessments were based on a challenge study conducted 28 days after vaccination (see Table 7).

The antiviral activity of *Moringa Oleifera* Plant as it contains alkaloids, flavonoids, saponins and tannins. The best antiviral activity of moringa extract concentration studied by several researchers because The aqueous seed extract of *M. oleifera* effect versus NDV was inspected according to Chollom et al. (2012) found that virus death was proportional to extract concentration and inversely related to antibody generation against NDV, indicating that *M. oleifera* seed aqueous extract possesses high antiviral activity against NDV in an ovo experiment. *M. oleifera* extract has nutritional value because it includes a lot of minerals for example calcium ions (Ferreira et al., 2008; Siddhuraju and Becker, 2003). These components could be the source of an increased immunological response. *M. oleifera*'s ability to boost immunological responses is due to the presence of growth factors such as cytokines, which stimulate both adaptive and innate immunity Davis and Kuttan (1998).

The effect of *Moringa Oleifera* leaf extract on hematological markers was studied. Most broiler chicken groups had leukocytosis, which could be linked to lymphocytosis caused by the vaccine's immunostimulatory impact (Latimer, 2011) a marked response to antigenic stimulation caused by viral infection (Doneley, 2018), and boosting the immune system's reaction Active *Moringa Oleifera* extract component stimulates immunological response via lymphocyte proliferation (Chollom et al., 2012) in vaccinated, infected, and *Moringa Oleifera* extract treated broiler chickens, respectively. In the vaccinated broiler group, monocytosis occurred as a result of an effective immune reaction of the body against the vaccine virus. (Simpraga et al., 2008) and transition of monocytes from bone marrow to the tissues as macrophages for mopping up of necrotic debris in infected broilers group Thomson (1984). In general, leukogram images demonstrated the highest degree of leukocytosis in broilers vaccinated and treated with *Moringa Oleifera* extract, possibly indicating a synergistic effect of the two to enhance the immune system of birds. White blood cell counts and immunoglobulin levels increased significantly when different doses of *Moringa Oleifera* were given Adedapo et al. (2005). Similarly, *Moringa Oleifera* leaf powder supplemented diets improved the immunological response of *O. niloticus* fry and prevented illness induced by *A. hydrophila*, according to (Abd El-Gawad et al., 2020).

Virus shedding is one of the parameters that evaluate the protective efficacy of the Newcastle virus after challenge. This study revealed a significant reduce in the virus shedding levels more in vaccinated and treated group than vaccinated group alone or treated group alone. The virus tiers were higher in oropharyngeal swabs than cloacal swabs in our experimental study. Tian and his colleague, 2005 registered the shedding of viral tiers from the trachea and observed that they were more than from the cloaca and it may be resulted in the efficiency of the replication for the upper respiratory tract after inoculation the challenge virus (Tian et al., 2005; Webster et al., 2006). The virus shedding was peaked at day 3 post challenge and remained significantly less in consecutive days post challenge while zero virus was detected in chickens on day 9th post challenge by titration in ECE-SPFs as reported by (Lee and Suarez, 2004; Webster et al., 1986). In the opposite side the shedding in the cloacal swabs were low it could be related to substances that make inhibition which present in fecal specimens that decrease PCR amplification and the limitation capacity of most

commercial RNA extraction kits to remove these inhibitors from clinical samples Das et al. (2009)

The phagocytic activity of *Moringa Oleifera* leaf extract on experimentally inoculated Hubbard chickens found that There is no difference in the increase in mean number of saccharomyces cerevisiae per macrophage cells in treated unvaccinated and vaccinated group, while in combined group with vaccines and plant show the highest phagocytic ability of saccharomyces cerevisiae per macrophage cells due to synergistic action to stimulate the immune system of birds. Gupta et al. (2010) reported that moringa extract pretreatment in mice blocked cyclophosphamide bone marrow suppressive effect on phagocytic activity.

## 5. Conclusion

The protection percentage and serological response in vaccinated chickens with Newcastle virus and treated with *Moringa Oleifera* leaf extract were improved and gives preferable results than vaccinated chickens only or treated chickens only so *Moringa Oleifera* leaf extract may be useful in the protection against Newcastle virus when used mixed with the vaccine.

## Ethics statement

The protocol was conducted according to the Committee of Animal Welfare and Research Ethics (protocol #ZU-IACU/2/F/9/2018) in Zagazig University, Egypt.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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